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-- Patent Application --
-- Attorney Docket No. 25,835.11 --

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: M. L. Collins, et al.

Serial No.: 08/238,080

Filing Date: May 3, 1994

Title: TARGET AND BACKGROUND CAPTURE METHODS WITH AMPLIFICATION FOR AFFINITY ASSAYS

Art Unit: 1807

Examiner: D. Rees

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail, postage prepaid, in an envelope addressed to: **The Assistant Commissioner for Patents, Washington, D.C. 20231**, on the date shown below.

LESLIE B. HENSON
(Printed Name)

Leslie B. Henson
(Signature)

OCTOBER 18, 1996
(Date of Deposit)

AMENDMENT

The Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Please amend the above-captioned application as follows.

IN THE SPECIFICATION:

Please delete the first full paragraph of the specification following line 4 on page 1 of the specification and insert in its place the following text:

--This application is a divisional application of U.S. Serial No. 08/400,657 filed March 8, 1995; which is a continuation application of U.S. Serial No. 08/257,469, filed June 8, 1994 and now abandoned; which is a continuation application of U.S. Serial No. 08/124,826, filed September 21, 1993 and now abandoned; which is a continuation application of US. Serial No. 07/946,749 filed September 17, 1992 and now abandoned; which is a continuation application of U.S. Serial No. 07/648,468 filed January 31, 1991 and now abandoned; which is a continuation-in-part application of US.

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Serial No. 07/136,920 filed December 21,1987 and now abandoned; and which is a continuation-in-part application of U.S. Serial No. 06/922,155 filed October 23, 1986 and now abandoned. The disclosures of Serial No. 07,136,920 and 06/922,155 are incorporated herein by reference.--

IN THE CLAIMS:

Please amend claims 43-45 as follows:

B1 43. (Amended) A method for detecting a target polynucleotide contained in a sample comprising the steps of:

(a) contacting the sample with a first support which binds to the target polynucleotide;

(b) substantially separating the first support and bound target polynucleotide from the sample;

B2 (c) amplifying the sample with a DNA polymerase;

(d) contacting the amplified target polynucleotide with a second support which binds to the amplified target polynucleotide and <u>also with</u> a labeled probe which binds to the amplified target polynucleotide; and

(e) detecting the presence of the <u>amplified</u> target polynucleotide.

B2 44. (Amended) A kit for detecting a target polynucleotide contained in a sample comprising:

(a) means for substantially separating the target polynucleotide from the sample;

(b) means for amplifying the target polynucleotide;

(c) means for binding the amplified target polynucleotide to a solid [medium] <u>support</u>; and

(d) means for labeling the amplified target polynucleotide.

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~~45.~~ (Amended) The kit of Claim ~~44~~ wherein:

(a) the means for substantially separating the target polynucleotide from the sample include a first support;

(b) the means for amplifying the target polynucleotide include a polymerase;

(c) the means for binding that amplified target polynucleotide to a solid [medium] support include a [second support] capture probe which binds to the solid support and to the amplified target polynucleotide; and

(d) a detector probe for labeling the amplified target polynucleotide.

Please add new claims 51-54 as follows:

51. The method of Claim 27 further comprising the steps of:

(a) contacting the support, probe and bound target with a second medium;

(b) releasing the target polynucleotide from the support and probe into the second medium; and

(c) substantially separating the support and probe from the medium.

52. The method of Claim 33 further comprising the steps of :

(a) contacting the support, probe and bound target polynucleotide with a second medium;

(b) releasing the target polynucleotide from the support and probe into the second medium; and

(c) substantially separating the support and probe from the medium.

53. A method for amplifying a target polynucleotide contained in a sample comprising the steps of:

(a) contacting the sample with reagent comprising a first nucleic acid probe which binds to the target to form a probe-target complex;

(b) contacting the sample with a support which binds to the first nucleic acid probe of the probe-target complex;

(c) substantially separating the support and bound probe-target complex from the sample;

(d) contacting the support and bound probe-target complex with a second medium;

(e) releasing the probe-target complex into the second medium;

(f) substantially separating the support from the second medium; and

(g) amplifying the target polynucleotide.

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54. A method for detecting a target polynucleotide contained in a sample comprising the steps of:

(a) contacting the sample with reagent comprising a first nucleic acid probe which binds to the target to form a probe-target complex;

(b) contacting the sample with a support which binds to the first nucleic acid probe of the probe-target complex;

(c) substantially separating the support and bound probe-target complex from the sample;

(d) contacting the support and bound probe-target complex with a second medium;

(e) releasing the probe-target complex into the second medium;

(f) substantially separating the support from the second medium;

(g) amplifying the target polynucleotide; and

(h) detecting the presence of the amplified target polynucleotide.

REMARKS

Status of the Application:

At present, claims 25-50 are under consideration in the application. All claims stand rejected under 35 USC § 112 and 35 USC § 103 by an office action mailed June 20, 1996.

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Priority of the Application:

Applicants were reminded by the Examiner that the application must contain in the first sentence a specific reference to all earlier filed applications which are to be relied upon for benefit of an earlier filing date. Applicants submit their amendment provides the required recitation.

Rejections Under § 112:

Claims 43 and 44 are rejected under 35 USC § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. Claim 43 is said to be indefinite for its recitation of the phrase "contacting the amplified target polynucleotide with a second support which binds to the target polynucleotide and detecting the presence of the amplified target polynucleotide" in step (d). The Examiner submits it is unclear whether the second support actually "binds" to the polynucleotide or only does so indirectly through a labeled probe. The Examiner further submits it is unclear whether the labelled probe is bound to the second support. The Examiner has requested clarification.

Claim 44 is said to be indefinite for its recitation of the phrase "to a solid medium include a second support" in step (c). The Examiner submits the phrase seems to be missing some words and requests clarification.

Rejections Under § 103:

Claims 25-42, 44 and 48-50 are rejected under 35 USC § 103 as unpatentable over Vary (U.S. Patent No. 4,851,331, filed May 16,1986) in view of Henson (sic Hanson) (EP 0 139 489, published Feb. 5, 1985). The Examiner submits Vary teaches a method for amplifying and detecting a target polynucleotide in a sample comprising amplifying a target polynucleotide, immobilizing said amplified polynucleotide on a support, separating the amplified polynucleotide on the support from the sample and detecting the amplified polynucleotides. Vary is said to teach the advantage of solid supports in "capturing a probe specifically and in high concentration with major other material not contributing to

nonspecific signal" (at column 4). The Examiner submits the method of Vary differs from the claimed invention in that Vary does not bind a target polynucleotide to a support or separate the target from the sample prior to amplification. The Examiner also admits Vary does not explicitly teach retrievable supports.

Further, the Examiner submits that Hanson teaches a nucleic acid detection method in which an enzyme labeled nucleic acid probe is hybridized to a sequence of interest as well as a biotinylated nucleic acid probe which may be immobilized on an avidin coated microparticle (i.e., a retrievable solid support) (citing the abstract). Moreover, the Examiner also submits that following separation of the support and bound target polynucleotide from the sample (page 4, para, 3), the target is detected by means of the labelled nucleic acid (page 5). The Examiner asserts Hanson teaches that the order of reaction between the single stranded nucleic acid and the probe and the support may be varied according to experimental needs (page 6).

The Examiner concludes that it would be *prima facie* obvious therefore, for one of ordinary skill in the art at the time the invention was made to modify the method of Vary by performing a separation/purification step prior to amplification given that techniques of capturing a target nucleic acid on a retrievable solid support were routinely performed in the art to isolate a target sequence from a complex biological sample and given that such methods were known to provide the benefit of increased sensitivity and lower background in nucleic acid detection assays. The Examiner further concludes that one of ordinary skill at the time the invention was made would be well aware that enriching for a desired target sequence in a population of sequences prior to a PCR amplification step would provide a more sensitive assay for such reasons and have been motivated to do so in view of the teachings of Vary to achieve the expected benefit of avoiding nonspecific signals that arise in PCR assays. The inclusion of reagents to be used in the method of Vary as modified by Hanson is further asserted to have been *prima facie* obvious to one of skill in the art at the time the invention was made in view of the conventionality of kits in the analytical arts and their well known benefits of providing standardized reagents in a convenient form.

Claims 25-50 are also rejected under § 103 as unpatentable over Vary in view of Henson (sic Hanson) and further in view of Rabbani (EP 0 159 719, published October 39, 1985). The Examiner asserts that Vary in view of Hanson meet all of the limitations of the claims except for the teachings of a second support. The Examiner further asserts, however, that Rabbani teaches a nucleic acid detection method which provides two probes as a means of detecting a single target wherein both probes may be labelled with particle and the particles may be macroparticles or microparticles (i.e., retrievable solid supports). Rabbani is further said to teach that an advantage to the method is to provide a highly sensitive, homogenous assay system. Thus, the Examiner concludes, it would have been *prima facie* obvious to one of ordinary skill to further modify the method taught by Vary in view of Hanson by using a probe labelled with a second support for the expected benefit of optimizing the sensitivity of the assay as taught by Rabbani. It would have been further *prima facie* obvious, the Examiner concludes, to provide the compositions of Hanson, Vary and Rabbani in a kit format for the expected benefit of provided for standardized reagents in a convenient format.

<u>Intervening Art:</u>

The Examiner has cited two additional references as intervening art. These are Longiaru (U.S. No. 5,232,829) and Urdea (U.S. No. 5,299,314). Although cited as intervening art, these references have not been cited in rejecting Applicants' claimed inventions.

Longiaru is cited for teaching a method for amplifying a target polynucleotide in a sample comprising the steps of contacting the sample with a first support which binds a target polynucleotide (in this example the first support is the surface of a microtiter well to which is bound a capture oligonucleotide) and substantially separating the support and bound target polynucleotide from the sample (washing the wells several times). Urdea is cited for teaching a method for amplifying and detecting a target polynucleotide by contacting the target with a capture probe attached to a solid support (i.e., the support binds to the target polynucleotide through the capture probe). The support and bound

target polynucleotide are then separated from the sample. The target polynucleotide is then amplified by PCR with a polymerase and the amplified product is separated from the reaction mixture. The Examiner submits the support may further be retrievable and the capture probe may be labelled.

Applicants' Amendments:

Applicants respectfully request entry of the amendments presented herein. Applicants submit the amendments add no new matter and place the application in condition for allowance. More particularly, as noted above, Applicants have amended their specification to recite specifically all earlier filed applications which are relied upon for benefit of Applicants' claimed priority date. Applicants have amended claim 43 to clarify the claim as suggested by the Examiner.

Claims 44-45 have been amended to clarify an ambiguity noted by the Examiner. The claims have been amended to clarify that the claimed kits include means for binding the amplified polynucleotide to the support and not to a solid medium (claim 44) and that the means for accomplishing this binding include a capture probe (claim 45). These limitations are clearly disclosed in the specification and figures of the application.

Applicants have added new claims 51-54 to emphasize additional features of Applicants' invention not previously claimed. New claims 51-52 depend from existing claims 27 and 33, respectively. New claims 53-54 are independent claims. The new claims add no new matter to the application. At the same time, however, the new claims are separately patentable over the claims now pending in the application.

The new claims resolve a particular aspect of the background noise problem not previously recognized by the art. More particularly, the new claims specifically address the problem resulting from nonspecific binding to the support used in an assay. The new claims feature the step of separating the support from the target following capture of the target and prior to amplification. Applicants have found that nonspecific binding of cellular

debris and other nucleic acids can result in production of impure amounts of amplified nucleic acids. As discussed previously, and is further discussed herein, nonspecific binding of nucleic acids to the solid support used in the assay can also result in amplification of unintended nucleic acids. If used in a hybridization assay, background noise results. When used to produce purified nucleic acid in quantity, impure product results. By separating the support from the target nucleic acid before amplification, amplification of unintended nucleic acid can be reduced substantially. Applicants submit this problem and this result have not been appreciated previously and so provide a separate basis for patentability.

[ ]

The features of new claims 51-54 are plainly described in Applicants' specification and so add no new matter to the application. For example, Applicants disclose that cellular debris will bind nonspecifically to solid supports at page 8, lines 13-15 of the specification. Applicants also disclose hybridization methods in which target is first captured on a solid support, separated from the support and then amplified at page 28, line 10 - page 29, line 35, Figures 4-6 and Examples 4-7 of the specification. Accordingly, the newly claimed methods add no new matter to the application.

Response to Rejections Under § 112:

Applicants traverse the rejections under § 112 as inappropriate or overcome in light of Applicants' amendments. Applicants submit the rejection to claim 43 is overcome in light of the amendment presented herein. Applicants have amended claim 43 to state the claimed method more clearly. The Examiner asserted it was not clear whether the claim intended the second support to bind to the target polynucleotide through a labeled probe. Applicants submit the amended claim clarifies that the second support is not intended to bind to the target through a labeled probe. Applicants note, however, that it is clearly contemplated that target nucleic acid may be bound to the support directly or indirectly through a capture probe. The use of indirect binding through a capture probe is disclosed in the application at pages 28-29, Figures 4-6 and Examples 4-7 of the specification.

Applicants have amended claims 44 and 45 to clarify incorrect recitations in the claims noted by the Examiner. In particular, the claims made reference to a "solid medium." Applicants acknowledge that recitation of the phrase "solid medium" was not contemplated as an element of Applicants' claimed invention. Rather, the appropriate element is a "solid support." Applicants' amendments make this change. Applicants have amended claim 45 to maintain consistency with claim 44.

Response to Rejections Under § 103:

Applicants traverse the rejections under § 103 because the references cited do not render the claimed inventions unpatentable under § 103. Applicants submit that the Examiner has ignored critical teachings of previously cited references (these purport to teach that the problem addressed by Applicants' invention did not exist) and other references previously made of record (these demonstrate that when those skilled in the art did come to recognize the problems addressed by Applicants' invention exist, they did not find Applicant's invention as a means to overcome those problems). Applicants submit that when the full record is considered, it is clear that Applicants' invention is patentable over the cited art.

As discussed more fully in Applicants' Preliminary Amendment, mailed December 5, 1995, Applicants' pending claims pertain to improved methods and kits for use in capturing, amplifying and detecting target molecules. The invention couples nucleic acid amplification techniques with noise reduction techniques to provide a detection method of great sensitivity. The invention also permits production of large amounts of purified target polynucleotides. More particularly, the invention enhances nucleic acid hybridization methods by combining target purification methods with target amplification methods.

Nucleic acid targets can be amplified using any of a variety of methods, including the polymerase chain reaction PCR. However, although PCR was introduced with the promise of great specificity for target amplification, the reality is that PCR is not as specific

as practitioners require. PCR will routinely amplify non-target polynucleotides in addition to target polynucleotides. While it may appear that the Examiner has made out a *prima facie* case that the claimed inventions are unpatentable as obvious over the cited art, Applicants submit that the Examiner's *prima facie* case fails when all teachings of record are fully considered.

Claims 25-42, 44 and 48-50 are rejected over Vary in view of Hanson. Vary is cited for teaching amplification and detection of a target polynucleotide, immobilizing the amplified polynucleotide on a support, separating the amplified polynucleotide and the support and detecting the amplified polynucleotide. Vary is also cited for teaching the advantage of solid supports in capturing a probe specifically and in high concentration with major other material not contributing to nonspecific signal. The Examiner cites Hanson for teaching nucleic acid detection wherein an enzyme labelled probe is hybridized to a target sequence and an immobilizeable, biotinylated probe. The Examiner further cites Hanson for teaching that target is detected by the labelled nucleic acid following separation of the support and bound target polynucleotide from the sample. The Examiner concludes it would have been obvious for the skilled artisan to modify the method of Vary by performing a separation/purification step prior to amplification given that the techniques for capturing target nucleic acid on a retrievable solid support were routinely performed and that such methods were known to provide increased sensitivity and lower background in nucleic acid assays. The Examiner also concluded that the skilled artisan would be aware that enriching for a desired target sequence prior to a PCR amplification step would provide a more sensitive assay and would be motivated to do so in view of the teachings of Vary to avoid nonspecific signals arising in PCR assays. Applicants submit these conclusions by the Examiner are belied by the evidence of record.

In particular, Applicants submit the Examiner's conclusions are belied by the fundamental teachings of previously cited Mullis and the numerous references cited by Applicants in their Preliminary Amendment. As discussed previously, Mullis teaches

improved sensitivity and ability to isolate specific nucleotide sequences. But Mullis also teaches:

> The present invention obviates the need for extensive purification of the product from a complicated biological mixture.

(Col, 5, lines 32-34). Mullis also taught:

> It is not necessary that the sequence to be amplified be present initially in a pure form; it may be a minor fraction of a complex mixture ... or a portion of a nucleic acid sequence due to a particular microorganism which organism might constitute only a very minor fraction of a particular biological sample.

(Col. 5, lines 49-56). Moreover, Schochetman, Vosberg, PCR Protocols, Coutlée and Miller, all cited in Applicants' Preliminary Amendment, demonstrate that years later practitioners of PCR found that PCR does not obviate the need for extensive purification of product as promised and does not render it unnecessary to initially purify a sequence to be amplified. Moreover, although they recognized the problem, these workers did not disclose or suggest Applicants' claimed method for solving it. Applicants submit it is improper for the Examiner to ignore these teachings, which relate directly to nucleic acid hybridization assays using amplification methods, and find Applicants' invention obvious by combining Hanson and Vary.. Hanson does not relate at all to amplification methodology and so is not properly combined with Vary to overcome the teachings of these other references which relate directly to amplification methods. Those with skill in the art canno reasonably be expected to credit the teachings of Hanson for amplification methods and ignore the contrary teachings of Mullis and the other references discussed herein. Accordingly, Applicants respectfully request that the rejections of claims 25-42, 44 and 48-50 as unpatentable over Vary and Hanson be withdrawn.

Claims 25-50 are further rejected under § 103 as unpatentable over Vary in view of Hanson and further in view of Rabbani. Vary and Hanson seem to be cited as in the previous rejection. However, these references do not disclose a second support. Claims

43 and 45-47 recite the use of a second support. Thus, Rabbani is cited for teaching the use of a second support and the resulting highly sensitive assay systems. Applicants submit that the teachings of Vary and Hanson are deficient as described before, and that Rabbani's disclosure of a second support does nothing to overcome these deficiencies. Rabbani does not disclose or discuss nucleic acid hybridization assays using amplification techniques, problems inherent thereto or Applicants' solution thereto. Thus, Rabbani cannot be combined with Vary and Hanson to render Applicants' claimed methods obvious.

Applicants' new claims are even more clearly distinguishable from the teachings of the cited art than are the inventions of the pending claims. As discussed supra, the new claims address a problem not previously appreciated by the prior art. The methods of claims 51-54 enable elimination of unintended amplification of undesirable subject matter that would otherwise result from nonspecific binding to the solid support used to capture the target polynucleotide. Applicants have found no teachings in the cited references or any other prior art which disclose or suggest this problem or Applicants' solution to this problem. Accordingly, Applicants submit that new claims 51-54 are separately patentable over presently pending claims 25-50.

Conclusion:

Applicants submit all rejections made in the office action mailed June 20, 1996 are inappropriate or overcome by Applicants' amendments. Applicants further submit that their amendments are appropriate and should be entered because they add no new matter and place the application in condition for allowance, which action is earnestly solicited.

Respectfully submitted,

AMOCO CORPORATION

By: Norval B. Galloway
Norval B. Galloway
Attorney for Applicants
Registration No. 33,595

Date: 10/18/96

AMOCO CORPORATION
3100 Woodcreek Drive
Downers Grove, Illinois 60515-5400
Phone: (630) 271-7417
Facsimile: (630) 271-7048
C:\DOCS\NBG\APPLN\2583511.AMD